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<b>(54) Title:</b> PREVENTION OF GRAFT-VERSUS-HOST DISEASE WITH T-CELLS INCLUDING POLYNUCLEOTIDES ENCODING NEGATIVE SELECTIVE MARKERS  <b>(57) Abstract</b>  A method of preventing graft-versus-host disease in a patient being treated for relapsed or persistent leukemia. The method comprises administering to a host allogeneic T-cells genetically engineered to include a polynucleotide encoding a negative selective marker. Prior to the occurrence of graft-versus-host disease, an interaction or chemotherapeutic agent is administered to the host, which kills the genetically engineered T-cells, thereby preventing the occurrence of graft-versus-host disease. Such method also may be employed in connection with the treatment of any disease or disorder wherein the treatment includes bone marrow ablation followed by the administration of a T-cell depleted bone marrow transplant.		

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PREVENTION OF GRAFT-VERSUS-HOST DISEASE WITH T-CELLS  
INCLUDING POLYNUCLEOTIDES ENCODING NEGATIVE  
SELECTIVE MARKERS

This invention relates to the prevention of graft-versus-host disease, or GVHD, in patients who have received allogeneic bone marrow transplants. More particularly, this invention relates to the prevention of graft-versus-host disease in patients who are suffering from relapsing or persistent leukemia, subsequent to an allogeneic T-cell depleted bone marrow transplant, by administering to such patients T-cells which include a polynucleotide encoding a negative selective marker, followed by the administration of an interaction agent or prodrug to kill the T-cells, in particular, those T-cells which are graft-versus-host reactive, before graft-versus-host disease develops.

In addition, this invention relates to the prevention of graft-versus-host disease in connection with the treatment of diseases or disorders wherein the treatment includes bone marrow ablation followed by the administration of an allogeneic bone marrow transplant, such as a T-cell depleted bone marrow transplant. In such a method, T-cells which include a polynucleotide encoding a negative selective marker also are administered to a patient, and an interaction agent or prodrug is administered to the patient to kill the T-

cells, in particular, those T-cells which are graft-versus-host reactive, before graft-versus-host disease develops.

#### Background of the Invention

Hematological malignancies, or leukemias, such as, for example, multiple myeloma (MM), chronic myelogenous leukemia (CML), acute myeloid leukemia (AML), and acute lymphoblastic leukemia (ALL), affect thousands of Americans per year. For example, multiple myeloma affects approximately 4 in 100,000 Americans per year. There were 12,800 new cases in 1993. Multiple myeloma comprises slightly more than 1% of all types of malignancies and slightly more than 10% of all hematological malignancies. (Barlogie, et al., JAMA, Vol. 268, pgs. 2946-2951 (1992); Barlogie, et al., Blood, Vol. 73, pgs. 865-879 (1989)). The median age at diagnosis is 62 and it is more common in men than in women. (Bortin, et al., Transplantation, Vol. 42, pg. 29 (1986).) Patients present with localized or disseminated disease, serum M-protein value of usually more than 3.0 g/dl and reduced values of uninvolved serum immunoglobulins. There is often a monoclonal light chain in the urine and skeletal lesions. The plasma cell labeling index,  $\beta 2$  microglobulin, and C-reactive protein (CRP) have been shown to be independent prognostic factors.

Allogeneic bone marrow transplantation has emerged as an effective treatment modality for selected patients with multiple myeloma (Bortin, et al., 1986). 268 patients have received allotransplants in different trials. (Barlogie, et al., Seminars in Hematology, Vol. 32, pgs. 31-44 (1995)). Fifty percent of the patients died within one year, about 40 percent achieved a complete response, and the four year projected event - free survival is approximately 35 percent.

Allogeneic bone marrow transplantation is thought to be curative, in part, because of an anti-tumor (i.e., graft-versus-myeloma, or GVM) effect derived from the adoptive transfer of immunocompetent cells in the donor graft. (Gale,

et al., The Lancet, Vol. 2, pg. 28 (1984); Bortin, et al., Nature, Vol. 67, pg. 722 (1979).) The anti-tumor effect which has been associated with graft-versus-host disease (GVHD), appears to be mediated by both T-cell and non-T-cell effector cell populations. GVHD, however, remains a major problem because of its morbidity and mortality.

In order to decrease the early mortality from allogeneic bone marrow transplantation, T-cell depletion has been employed. T-cells may be depleted from the allograft by any of several techniques, including density gradient centrifugation, soybean lectin agglutination and E-rosette formation, centrifugal elutriation, cytotoxic drugs or corticosteroids, anti-T-cell monoclonal antibodies, and positive selection of CD34+ cells. (Champlin, Journal of Hematotherapy, Vol. 2, pgs. 27-42 (1993); Reisner, et al., The Lancet, Vol. 2, pgs. 327-331 (1981); Waldmann, et al., The Lancet, Vol. 2, pgs. 483-486 (1984); Antin, et al., Blood, Vol. 78, pgs. 2139-2149 (1991); Soiffer, et al., J. Clin. Oncol., Vol. 10, pgs. 1191-1200 (1992).) Of the techniques used currently to deplete T-cells from a marrow graft, the most efficient is that of Reisner, et al. (1981). This technique, which consistently removes 2.5-3.0 log<sub>10</sub> clonable T-cells, first employs differential agglutination with soybean lectin to remove mature leukocytes, including T-cells, B-cells, monocytes, and granulocytes, followed by E-rosette depletion for removal of residual T-cells. This technique has been used to treat a population of over 200 HLA-matched related transplants given to leukemia patients. In this population, the incidence of Grade II acute GVHD has been 5%, and Grades III and IV acute GVHD have not been observed. (O'Reilly, et al., Bone Marrow Transplantation, Vol. 3, pgs. 3-6 (1988).)

Although T-cell depletion decreases the incidence of GVHD, T-cell depletion also increases the risk of early

relapse, incomplete immunological reconstitution, graft failure, and Epstein-Barr Virus-related lymphoma.

The risk of relapse has been documented best in chronic myelogenous leukemia. (Marmont, et al., Blood, Vol. 78, pgs. 2120-2130 (1991); Goldman, et al., Ann. Int. Med., Vol. 108, pgs. 806-814 (1988).) Direct evidence of a graft-versus-leukemia (GVL) effect has been demonstrated in patients with recurrent chronic myelogenous leukemia after a T-cell depleted bone marrow transplant by infusion of peripheral blood mononuclear cells from the bone marrow donor without any additional chemotherapy or radiotherapy, resulting in at least a 6 log<sub>10</sub> kill of leukemic cells. (Drobyski, et al., Blood, Vol. 82, pgs. 2310-2318 (1993); Sullivan, et al., N. Engl. J. Med., Vol. 320, pgs. 828-834 (1989); Porter, et al., N. Engl. J. Med., Vol. 300, pgs. 100-106 (1994); Slavin, et al., Bone Marrow Transplantation, Vol. 6, pgs. 155-161 (1990); Sosman, et al., Amer. J. Pediatr. Hem./Onc., Vol. 15, pgs. 185-195 (1993).) Although infused peripheral blood mononuclear cells can induce complete and long-term remission in such patients, such therapy may be associated with GVHD. (Bar, et al., J. Clin. Oncol., Vol. 11, pg. 513 (1993); Drobyski, et al., 1993; Slavin, et al., 1990.)

Allogeneic bone marrow transplantation following T-cell depletion also has been associated with increased incidence of bone marrow engraftment failure. (Beatty, et al., N. Engl. J. Med., Vol. 315, pgs. 765-771 (1985); Hale, et al., Transplantation, Vol. 45, pgs. 753-759 (1988); Patterson, et al., Br. J. Hematol., Vol. 63, pgs. 221-230 (1986).) In a murine model for allogeneic bone marrow transplantation, durable engraftment was observed only after addition of potent immunosuppressive treatment with total body irradiation (TBI) in the conditioning regimen to deplete the host's T-cells. (Lapidot, et al., Blood, Vol. 73, pg. 2025 (1989)). In human allotransplantation, the same problem is

overcome by adding thiotepa and anti-thymocyte globulin to the conditioning regimen.

The immune deficiency after a T-cell depleted bone marrow transplant is very pronounced as the consequence of the intensive conditioning regimen and the absence of mature donor T-cells in the transplant. It is probably prolonged by larger differences in minor histocompatibility antigens between donor and patient.

One of the consequences of the severe immune deficiency in T-cell depleted bone marrow transplants is the increased risk of Epstein-Barr Virus (EBV) lymphoproliferative disorders. (Shapiro, et al., Blood, Vol. 71, pgs. 1234-1243 (1988); Zutter, et al., Blood, Vol. 72, pgs. 520-522 (1988).) These lymphoproliferative disorders are often unresponsive to standard forms of therapy. The Memorial Sloan-Kettering Bone Marrow Transplantation Group has reported on 7 patients developing lymphoproliferative disorder who were treated with donor lymphocyte infusion. (Papadopoulos, et al., Blood, Vol. 82, supp. 1:214a (1993).) All patients were recipients of T-cell depleted transplants (4 related, 3 unrelated). The lymphoproliferative disorders developed within 6 months post transplantation. Biopsy specimens demonstrated a diffuse large cell lymphoma. Four evaluable specimens were found to be of donor cell origin. EBV DNA was detected in 5 of 5 evaluable samples by PCR. The patients received donor leukocytes at doses providing  $0.2-1.0 \times 10^6$  CD3+ cells/kg of patient weight. Complete pathologic and/or clinical responses were observed in all 5 patients. These responses were documented pathologically by 8-21 days post-infusion. Clinical remissions were achieved within 14-30 days and have been sustained without other therapy in the 3 surviving patients. Two patients died 8 days and 16 days after infusion from sepsis and interstitial pneumonia, respectively. Autopsy did not reveal any evidence of residual lymphoma. The 3 surviving patients developed GVHD.

Tiberghien, et al., in a series of abstracts and in a journal article, (Tiberghien, et al., Proceedings of the American Association for Cancer Research, Vol. 34, pg. 338, abstract 2011 (March 1993); Tiberghien, et al., J. Cell. Biochem., Supp. 17E, pg. 234, abstract SZ223 (1993); Tiberghien, et al., Nouv. Rev. Fr. d'Hematol, Vol. 35, pg. 329 (1993); Tiberghien, et al., First Meeting of the European Working Group on Human Gene Transfer and Therapy, Abstract Book, pg. 44 (November 19, 1993); Tiberghien, et al., Blood, Vol 84, No. 4, pgs. 1333-1341 (August 15, 1994)) disclose the transduction of T-cells with a retroviral vector including the Herpes Simplex Virus thymidine kinase gene. Ganciclovir treatment of the transduced T-cells resulted in a growth inhibition of these cells which was greater than 80%. The ganciclovir had no effect on control (non-transduced) T-cells. Tiberghien, et al., then state that T-cells may be transduced with the Herpes Simplex Virus thymidine kinase (TK) gene ex vivo, and then be administered to a patient with hematopoietic stem cells. If the patient develops GVHD, ganciclovir may be administered to the patient in order to deplete the transduced T-cells.

#### Summary of the Invention

It is an object of the present invention to treat patients with relapsed or persistent leukemia after a T-cell depleted allogeneic bone marrow transplant. Such patients are given T-cells which have been genetically engineered to include a polynucleotide encoding a negative selective marker. After the cells have remained in the patient for an amount of time sufficient to provide a therapeutic effect, an interaction agent or prodrug is administered to the patient, whereby the genetically engineered T-cells are killed, and the development of GVHD is prevented. Alternatively, if GVHD develops before the predetermined time of administration of the interaction agent, the interaction agent may be



administered to the patient at the time of development of GVHD, whereby the GVHD is treated through the killing of the transduced T-cells.

It is another object of the present invention to prevent graft-versus-host disease in patients being treated for diseases or disorders wherein the treatment includes bone marrow ablation followed by the administration of a T-cell depleted bone marrow transplant. Such diseases or disorders include, but are not limited to, solid tumor malignancies, and acquired or genetic immunologic or hematopoietic diseases. During the treatment, T-cells genetically engineered with a polynucleotide encoding a negative selective marker are administered to the patient. After the cells have remained in the patient for an amount of time sufficient to provide a therapeutic effect, an interaction agent or prodrug is administered to the patient, whereby the genetically engineered T-cells are killed, thereby preventing graft-versus-host disease.

#### Detailed Description of the Invention

In accordance with an aspect of the present invention, there is provided a method of preventing graft-versus-host disease in a host that is being treated for a disease or disorder which is treatable by administering T-cells to a host, such as, for example, a relapsed or persistent leukemia. The method comprises administering to a host T-cells genetically engineered to include a polynucleotide encoding a negative selective marker or "suicide" gene. The cells are administered in an amount effective and remain in the host for a period of time effective to provide a therapeutic effect in the host. After the T-cells have remained in the host for a period of time sufficient to provide a therapeutic effect in the host, and prior to the occurrence of graft-versus-host disease, an interaction agent or prodrug is administered to the host. The interaction agent is administered to the host in an amount effective to

kill the genetically engineered T-cells, in particular, those T-cells which are graft-versus-host reactive, i.e., capable of providing a graft-versus-host effect, thereby preventing the occurrence of graft-versus-host disease in the host.

The term "polynucleotide" as used herein means a polymeric form of nucleotide of any length, and includes ribonucleotides and deoxyribonucleotides. Such term also includes single- and double-stranded DNA, as well as single- and double-stranded RNA. The term also includes modified polynucleotides such as methylated or capped polynucleotides.

The polynucleotide encoding the negative selective marker may be contained within an appropriate expression vehicle which is transduced into the T-cells. Such expression vehicles include, but are not limited to, eukaryotic vectors, prokaryotic vectors (such as, for example, bacterial vectors), and viral vectors.

In one alternative embodiment, the polynucleotide encoding the agent, or an expression vehicle containing the polynucleotide encoding the agent, is contained within a liposome.

In one preferred embodiment, the expression vehicle is a viral vector. Viral vectors which may be employed include DNA virus vectors (such as adenoviral vectors, adeno-associated virus vectors, Herpes Virus vectors, and vaccinia virus vectors), and RNA virus vectors (such as retroviral vectors). When an RNA virus vector is employed, in constructing the vector, the polynucleotide encoding the negative selective marker is in the form of RNA. When a DNA virus vector is employed, in constructing the vector, the polynucleotide encoding the negative selective marker is in the form of DNA.

In one embodiment, the viral vector is a retroviral vector. Examples of retroviral vectors which may be employed include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, and vectors derived from

retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus. The vector is preferably an infectious, replication incompetent retrovirus particle.

Retroviral vectors are useful as agents to mediate retroviral-mediated gene transfer into eukaryotic cells. Retroviral vectors are generally constructed such that the majority of sequences coding for the structural genes of the virus are deleted and replaced by the gene(s) of interest. Most often, the structural genes (*i.e.*, gag, pol, and env), are removed from the retroviral backbone using genetic engineering techniques known in the art.

The removal of the gag, pol and env genes results in a vector backbone, comprised of a 5' LTR, a packaging signal, one or more cloning sites, into which the heterologous gene or genes of interest can be introduced, and a 3' LTR. The preferred vector backbone is the G1 vector backbone, which is disclosed in McLachlin, et al., Virology, 195:1-5 (1993) and in PCT Patent Application No. WO 91/10728 for "Novel Retroviral Vectors," published on July 25, 1991.

The heterologous gene or genes are incorporated into the proviral backbone by standard techniques to form the retroviral vector. Techniques for the preparation of retroviral vectors are disclosed in PCT application WO 91/10728 as well as the following articles: Armentano, et al., J. Virol., 61:1647-1650 (1987), Bender, et al., J. Virol., 61:1639-1646 (1987), and Miller, et al., Biotechniques, 7:980-990 (1989). The most straightforward constructions are ones in which the structural genes of the retrovirus are replaced by a single gene which then is transcribed under the control of the viral regulatory sequences within the long terminal repeat (LTR). Retroviral vectors have also been constructed which can introduce more than one gene into target cells. Usually, in such vectors

one gene is under the regulatory control of the viral LTR, while the second gene is expressed either off a spliced message or is under the regulation of its own, internal promoter. Suitable promoters include the SV40 promoter, the human cytomegalovirus (CMV) promoter, the beta-actin promoter, the alpha fetoprotein promoter, and any promoter naturally associated with any heterologous gene of interest. Additionally, a polycistronic vector can be created by using an internal ribosome entry site.

The retroviral vectors may be in the form of a plasmid, a segment of viral RNA, or a segment of proviral DNA. For the present invention, the preferred retroviral vector is G1TK1SvNa, which is disclosed in PCT Patent Application No. WO 95/06486, published on March 9, 1995, entitled Treatment of Human Tumors by Genetic Transformation of Human Tumor Cells.

The retroviral vector is introduced into a packaging cell to form a producer cell. Packaging cells provide the gag, pol, and env genes in trans, which permits the packaging of the retroviral vector into a recombinant retrovirus that is infectious but replication defective. The vectors are transferred into the packaging cells by standard gene transfer techniques, which include transfection, transduction, calcium phosphate precipitation, electroporation, and liposome-mediated DNA transfer. Examples of packaging cells that may be used include, but are not limited to, the PE501, PA317, Psi-2, Psi-AM, PA12, T19-14X, VT-19-17-H2, Psi-CRE, Psi-CRIP, GP+E-86, GP+envAM12, PG13, and DAN cell lines. A preferred producer cell line for the present invention for the production of recombinant retrovirus is the producer cell line designated PA317/G1TK1SvNa, which is disclosed in PCT application WO 95/06486.

Negative selective markers include, but are not limited to, viral thymidine kinases such as Herpes Simplex Virus

thymidine kinase, cytomegalovirus thymidine kinase, and varicella-zoster virus thymidine kinase; xanthine-guanine phosphoribosyl transferase; and cytosine deaminase.

The retroviral vectors containing the polynucleotide encoding the negative selective marker are transduced into T-cells. In general, from about  $10^6$  to about  $10^9$ , preferably from about  $10^7$  to about  $10^8$  T-cells are transduced with the retroviral vectors, which may be contained in from about 2 ml to about 500 ml of retroviral supernatant having a titer of from about  $10^5$  cfu/ml to about  $10^9$  cfu/ml, preferably from about  $2 \times 10^6$  cfu/ml to about  $1 \times 10^8$  cfu/ml.

Once the T-cells are transduced with the retroviral vectors including the polynucleotide encoding the negative selective marker, the T-cells are administered to a host suffering from a relapsed or chronic leukemia. The host is an animal host, and in particular is a mammalian host, including human and non-human primate hosts. The transduced T-cells are administered by means known to those skilled in the art, including intravascular administration, such as intravenous or intraarterial administration; or by intraperitoneal administration. In one embodiment, the transduced T-cells are administered as a bolus infusion. The transduced T-cells are administered in an amount effective to provide a therapeutic effect, i.e., in an amount effective to treat the relapsed or persistent leukemia in the host. In general, the transduced T-cells are administered in an amount of from about  $10^5$  cells/kg to about  $10^9$  cells/kg, preferably from about  $2 \times 10^5$  cells/kg to about  $1 \times 10^7$  cells/kg.

The transduced T-cells are administered in conjunction with an acceptable pharmaceutical carrier, such as, for example, saline solution, or aqueous buffers, such as phosphate buffers, Tris buffers, Plasmalyte A (Baxter), or lactated Ringer's solution. The selection of a suitable pharmaceutical carrier is deemed to be apparent to those skilled in the art from the teachings contained herein.

Alternatively, the transduced T-cells may be frozen in an acceptable cryopreservation medium (such as, for example, a medium including phosphate buffered saline, 5% DMSO, and human albumin) until the cells are administered to the host. Prior to administration, the cells and medium are thawed, and the cells and medium are administered to the host upon thawing.

After a period of time sufficient to allow the transduced T-cells to provide a therapeutic effect in the host, but prior to the development of graft-versus-host disease, an interaction or chemotherapeutic agent is administered to the host in an amount effective to kill the transduced T-cells, and in particular, to kill transduced proliferating graft-versus-host-reactive T-cells, i.e., T-cells which are capable of providing a graft-versus-host effect, thereby preventing the development of graft-versus-host disease.

Preferably, the interaction agent is administered when the T-cells which are graft-versus-host reactive (whereby such T-cells which are capable of providing a graft-versus-host effect through the recognition of MHC Class I antigens of the host cells) are in a proliferative phase, and a portion of T-cells such as, for example, some of those T-cells which provide a graft-versus-leukemia effect or which provide an anti-viral effect, are in a quiescent or non-proliferative phase. The interaction agent, or prodrug, when administered at such a time, will provide for the killing of the proliferating T-cells which are capable of providing a graft-versus-host effect, whereas the non-proliferating T-cells, which include a portion of T-cells which provide a graft-versus-leukemia effect or an anti-viral effect, will survive the administration of the interaction agent or prodrug. Thus, the majority of the T-cells responsible for causing GVHD are ablated preferentially, while other T-cells capable of providing a graft-versus-leukemia effect or an

anti-viral effect remain. In general, the interaction agent is administered at a period of time of from about 10 days to about 50 days, preferably from about 14 days to about 28 days, more preferably at 21 days, after the administration of the transduced T-cells.

When the negative selective marker is a viral thymidine kinase, such as those hereinabove described, the interaction or chemotherapeutic agent or prodrug preferably is a nucleoside analogue, for example, one selected from the group consisting of ganciclovir, acyclovir, and 1-2-deoxy-2-fluoro- $\beta$ -D-arabinofuranosil-5-iodouracil (FIAU). Such interaction agents are utilized efficiently by the viral thymidine kinases as substrates, and such interaction agents thus are incorporated lethally into the DNA of the transduced T-cells expressing the viral thymidine kinases, and in particular, proliferating T-cells which are graft-versus-host reactive, thereby resulting in the death of the transduced T-cells.

When the negative selective marker is cytosine deaminase, a preferred interaction agent or prodrug is 5-fluorocytosine. Cytosine deaminase converts 5-fluorocytosine to 5-fluorouracil, which is highly cytotoxic. Thus, the transduced T-cells which express the cytosine deaminase gene convert the 5-fluorocytosine to 5-fluorouracil and are killed.

The interaction agent or prodrug is administered in an amount effective to provide for the death of the transduced T-cells. The interaction agent is administered preferably by systemic administration, such as by intravenous administration. In general, the interaction agent is administered in an amount of from about 2 mg/kg/day to about 10 mg/kg/day, preferably about 10 mg/kg/day, for a period of from about 3 to 18 days, preferably for about 5 days. In a preferred embodiment, the interaction agent or prodrug is administered in an amount of 5 mg/kg every 12 hours for a period of 5 days.

In the event the host develops graft-versus-host disease prior to the desired time that one wishes to kill the transduced T-cells, the interaction agent is administered upon the development of the graft-versus-host disease, thereby treating the graft-versus-host disease in the host.

Thus, the method of the present invention enables one to treat a patient suffering from a relapsing or chronic hematological malignancy or leukemia by administering T-cells to the patient, and to prevent the occurrence of graft-versus-host disease by killing the T-cells after the T-cells have provided a desired therapeutic effect. Leukemias which may be treated with the transduced T-cells include, but are not limited to, multiple myeloma (MM), myelodysplastic syndrome, chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma, Hodgkin's disease, and myelofibrosis.

An advantage of the present invention is that the T-cells which are genetically engineered to include a polynucleotide encoding a negative selective marker are safer than T-cells which are not genetically engineered to be employed in the treatment of relapsed or chronic leukemia because the major cause of mortality and morbidity, graft-versus-host disease, may be prevented by the administration of a prodrug or interaction agent. Because graft-versus-host disease is a consequence of the degree of mismatch between the donor and patient, the method of the present invention expands the number of patients that are eligible for an allogeneic bone marrow transplant to include older patients and more genetically disparate donors, including HLA non-identical siblings and matched, unrelated donors.

In addition, the timing of the administration of the interaction agent or prodrug is such that the majority of the GVHD reactive cells (i.e., the T-cells that are responsible for causing GVHD) are ablated preferentially, thus leaving



the patient with the greater part of the immunologic T-cell repertoire. It is likely that at least a part of the graft-versus-leukemia reactive cells and T-cells which are reactive with antigens other than those associated with GVHD, will survive the administration of the interaction agent or prodrug, thus helping to decrease the frequency of EBV lymphomas and infections.

In addition to the treatment of leukemia, the method hereinabove described may be applied to the treatment of other diseases and disorders. For example, the method may be applied to the treatment of solid tumor malignancies, especially in cases where the tumor has metastasized and tumor cells are found in the bone marrow. During such treatment, patients would undergo a high dose chemotherapy treatment with or without total body irradiation followed by rescue with an allogeneic T-cell depleted bone marrow transplant. T-cells genetically engineered with a polynucleotide encoding a negative selective marker or suicide gene would be administered to the patient as hereinabove described, and GVHD would be prevented by the administration of the interaction agent or prodrug. In this embodiment, the T-cells provide a graft-versus-tumor (GVT) effect. Solid tumor malignancies which may be treated in accordance with this method include, but are not limited to, breast cancer, neuroblastoma, testicular carcinoma, ovarian and uterine carcinomas, and soft tissue sarcomas.

In addition, the hereinabove described method of the present invention may be employed in the treatment of any acquired or genetic immunologic or hematopoietic disease in which treatment thereof includes bone marrow ablation followed by the administration of a T-cell depleted bone marrow transplant. Such diseases include, but are not limited to, AIDS, severe combined immune deficiency (SCID), Wiskott-Aldrich syndrome, disorders of lymphocyte function, disorders of myeloid function, aplastic anemia, Fanconi's

anemia, thalassemia, sickle cell anemia, enzyme deficiencies (including Gaucher's disease, the mucopolysaccharidoses, and the leukodystrophies), and osteopetrosis, a disease which is characterized by a deficiency in bone marrow cells which break down older bone. In these diseases, there is no graft-versus-leukemia or graft-versus-tumor effect. The genetically engineered T-cells, in this embodiment, would be responsible for improved bone marrow engraftment, and for decreasing the incidence of EBV lymphoma. GVHD is prevented by administration of the prodrug or interaction agent.

Thus, in accordance with another aspect of the present invention, there is provided a method of treating a disease or disorder in a host (which may be a mammalian host, including human and non-human primate hosts) wherein treatment of the disease or disorder in the host includes ablating the bone marrow of the host, followed by the administration of a T-cell depleted bone marrow transplant to the host. The method comprises ablating the bone marrow of the host. A T-cell depleted bone marrow transplant then is administered to the host. The host then is administered T-cells genetically engineered to include a polynucleotide encoding a negative selective marker, which may be selected from those hereinabove described. Prior to the occurrence of graft-versus-host disease, an interaction agent or prodrug (such as those hereinabove described) is administered to the host in an amount effective to kill the genetically engineered T-cells in the host.

Diseases or disorders which may be treated in accordance with this method include, but are not limited to, those hereinabove described. The polynucleotide encoding the negative selective marker may be contained in an appropriate expression vehicle such as those hereinabove described, including viral vectors such as retroviral vectors.

The genetically engineered T-cells may be administered to the host in an amount effective to provide a therapeutic

effect. Such amount may be as hereinabove described. The exact amount of genetically engineered T-cells to be administered is dependent upon a variety of factors, including the age, weight, and sex of the patient, the disease or disorder being treated, and the extent and severity thereof.

After a period of time sufficient to allow the genetically engineered T-cells to provide a therapeutic effect in the host, but prior to the development of graft-versus-host disease, the prodrug or interaction agent is administered in an amount, which may be as hereinabove described, which is effective in killing the genetically engineered T-cells, and in particular, those genetically engineered T-cells capable of providing a graft-versus-host effect, thereby preventing the development of graft-versus-host disease. The period of time after the administration of the genetically engineered T-cells at which the interaction agent is administered may be as hereinabove described.

#### EXAMPLES

The invention now will be described with respect to the following examples; however, the scope of the present invention is not intended to be limited thereby.

##### Example 1

Administration of T-cells transduced with a retroviral vector including a Herpes Simplex Virus thymidine kinase gene to patients suffering from persistent or relapsing multiple myeloma, followed by administration of ganciclovir

##### Collection of donor lymphocytes

50 to 100 ml of donor blood are collected, yielding at least  $2.5 \times 10^7$  cells. The blood is mixed with an equal volume of sterile phosphate buffered saline (PBS) and subjected to gradient separation using Ficoll (Lymphoprep, Nycomed), and the mononuclear cells are separated. The cells then are washed in sterile PBS three times. The cells are

resuspended in serum-free AIM-V medium containing 100 units/ml penicillin and 100 µg/ml streptomycin (Pen-Strep, Gibco-BRL) at a density of  $1 \times 10^6$  cells/ml with 1 µg/ml anti-CD3 antibody (Orthoclone OKT3, Ortho-Biotech), and incubated at 37°C in 5% CO<sub>2</sub> for 24 hours. Recombinant human Interleukin-2 (Chiron Corporation) is added at 1,500 units/ml and cells are cultured at 37°C in 5% CO<sub>2</sub> for a period of up to 7 days. When the lymphocytes are in the exponential proliferation phase, they are transduced with the retroviral vector.

Transduction of lymphocytes with G1TK1SvNa

Approximately  $1 \times 10^8$  lymphocytes are transduced with the retroviral vector G1TK1SvNa, which includes the Herpes Simplex Virus thymidine kinase (TK) gene and a neomycin resistance gene. Such transduction is accomplished by adding to the lymphocytes 100 ml of viral supernatant containing from  $2 \times 10^8$  cfu to  $1 \times 10^9$  cfu of the retroviral vector. The retroviral vector G1TK1SvNa is described further in PCT Application No. WO95/06486, published March 9, 1995. Fresh viral supernatant is added daily for a total of three days, along with additional amounts of Interleukin - 2 and protamine sulfate to achieve final Interleukin - 2 concentrations of 1,500 units/ml and protamine sulfate at 5 µg/ml. The cells are incubated at 37°C, 5% CO<sub>2</sub> for 24 hours after each addition of supernatant and protamine sulfate.

Twenty-four hours after the third transduction, the cells are resuspended at a density of  $1 \times 10^6$  cells/ml in fresh medium containing Pen-Strep and 1,500 units/ml of Interleukin-2 and incubated for 3 to 5 days. Geneticin then is added at an active concentration of 300 µg/ml, and cells are selected for three days, are centrifuged and then are resuspended in AIM-V medium containing Interleukin-2 and cultured until an adequate number of cells are obtained. In general, the cells are cultured for at least an additional four days.

The efficiency of transduction and selection is measured by a ganciclovir killing assay. One to two percent of the transduced cells are sampled for the ganciclovir killing assay. (A minimum of  $2 \times 10^6$  cells are required to perform the assay.) The assay is performed by suspending transduced lymphocytes in 5.0 ml of AIM-V (Gibco-BRL) medium containing 1,500 units/ml recombinant Interleukin-2 in tissue culture flasks. A total of three flasks are prepared. Ganciclovir is added to two of the flasks, one at a concentration of  $20 \mu\text{M}$  and the second at  $50 \mu\text{M}$ . The third flask is used as a normal control. The cells are incubated at  $37^\circ\text{C}$ , in 5%  $\text{CO}_2$  for five days. The percentage of live cells (trypan blue negative) are counted after 3 and 5 days of culture in ganciclovir.

If the ganciclovir kill at a concentration of  $50 \mu\text{M}$  is less than 85%, the cells are reselected in Geneticin as described above. Cells are cryopreserved and infused only if the ganciclovir kill is greater than or equal to 85%. In order to provide enough cells for two infusions (if needed), an adequate number of cells initially is collected, expanded, and cryopreserved for each patient. The transduced lymphocytes also are studied for subset analysis (CD3, CD4, CD8, CD19, and CD56) by FACS prior to infusion.

Infusion of transduced lymphocytes and administration of ganciclovir.

Infusion of the transduced lymphocytes is given to patients who have undergone an allogeneic bone marrow transplant with T-cell depletion if they show evidence of persistent disease 90 days after transplantation, or measurable relapse at anytime. Patients with persistent disease have greater than 20% plasma cells in a bone marrow aspirate or biopsy, and/or presence of serum M-component, and no reduction in the M-component in the last 6 weeks, and/or Bence Jones Proteinuria with no reduction in the last 6 weeks.

Each of three patients receives the transduced lymphocytes in an amount of  $1 \times 10^6$  lymphocytes/kg. One to 50 ml of lymphocytes is administered as a bolus infusion, and each patient is observed for 4 hours thereafter.

Twenty-one days after the patients are given the transduced lymphocytes, or if graft-versus-host disease (GVHD) develops earlier, the patients are given ganciclovir (Cytovene, Syntex Corporation, Palo Alto, California) by intravenous infusion in an amount of 5 mg/kg every 12 hours daily for 5 days. Patients with a complete response or a partial response with continued reduction in measurable disease at forty-two days after the injection of the transduced lymphocytes will not receive a second lymphocyte infusion until disease plateau or progression is observed.

Complete response should include, for a minimum of two weeks, the following: (i) absence in urine and serum of M-components by immunofixation; (ii) bone marrow which is adequately cellular (i.e. >20%) with less than 3% plasma cells by immunostaining; (iii) no elevation in serum calcium level; and (iv) no new bone lesions nor enlargement of existing lesions. Partial response requires, for at least 4 weeks, the following: (i) reduction of serum M-component by at least 5%; (ii) reduction of urinary M-protein to less than 200 mg/24 hrs. and to less than 10% of pretreatment values; and (iii) no new lytic bone lesions or soft tissue plasmacytoma. Improvement also should include a reduction of serum paraprotein level and urinary light chain excretion by 25-50% compared with baseline values, and a decrease of bone marrow infiltration by plasma cells by 25-50% compared with baseline values.

Patients with a partial response without a continued decline in M-protein will receive, forty-two days after the first infusion of transduced lymphocytes, the same number of transduced lymphocytes as during the first infusion.

Ganciclovir is not administered unless the patient develops GVHD.

For this example, dose levels are as follows:

<u>DOSE LEVEL</u>	<u>LYMPHOCYTE INFUSION DOSE</u> <u>(LYMPHOCYTES/KG)</u>
I	$1 \times 10^6$
II	$5 \times 10^6$
III	$1 \times 10^7$
IV	$2 \times 10^7$
V	$5 \times 10^7$

Also, for purposes of this example, GVHD is graded as follows:

CLINICAL GRADING OF ACUTE GVHD

<u>GRADE</u>	<u>SKIN</u>	<u>LIVER</u>	<u>GUT</u>	<u>FUNCTIONAL IMPAIRMENT</u>
0	0	0	0	0
I	+ to ++	0	0	0
II	+ to +++	+ or +		+
III	++to+++	++to+++	++to+++	++
IV	++to++++	++to++++	++to++++	+++

**STAGING BY ORGAN SYSTEM**

<b>SKIN</b>	<b>% OF BODY SURFACE AFFECTED</b>
+	<25
++	25-50
+++	>50
++++	BULLAE, DESQUAMATION

<b>LIVER</b>	<b>BILIRUBIN (MG%)</b>
+	2 - 3
++	3.1 - 6
+++	6.1 - 15
++++	>15

<b>GUT</b>	<b>DIARRHEA (ML/DAY)</b>
+	>500
++	>1,000
+++	>1,500
++++	PAIN/ILEUS

If the first three patients do not achieve a complete response or partial response, and do not develop Grade III or Grade IV GVHD, the dose of lymphocytes is escalated from  $1 \times 10^6$  lymphocytes/kg to  $5 \times 10^6$  lymphocytes/kg (i.e., dose level II).

The treatment plan for each dose level is identical to the one described above for dose level I (i.e.,  $1 \times 10^6$  lymphocytes/kg). Six additional patients are entered at the dose level at which one of three patients responds, or at one dose below the level at which toxicity is observed.

The disclosure of all patents, publications (including published patent applications), and database entries referenced in this application are specifically incorporated



herein by reference in their entirety to the same extent as if each such individual patent, publication, and database entry were specifically and individually indicated to be incorporated by reference.

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced other than as particularly described and still be within the scope of the accompanying claims.

## WHAT IS CLAIMED IS:

1. A method of preventing graft-versus-host disease in a host being treated for relapsed or persistent leukemia, comprising:

administering to a host T-cells genetically engineered to include a polynucleotide encoding a negative selective marker; and

administering to said host, prior to the occurrence of graft-versus-host disease, an interaction agent, said interaction agent being administered in an amount effective to kill genetically engineered T-cells capable of providing a graft-versus-host effect in said host through interaction of said interaction agent with said negative selective marker.

2. The method of Claim 1 wherein said negative selective marker is selected from the group consisting of thymidine kinases, xanthine-guanine phosphoribosyl transferase, and cytosine deaminase.

3. The method of Claim 2 wherein said negative selective marker is selected from the group consisting of Herpes Simplex Virus thymidine kinase, cytomegalovirus thymidine kinase, and varicella-zoster virus thymidine kinase.

4. The method of Claim 3 wherein said negative selective marker is Herpes Simplex Virus thymidine kinase.

5. The method of Claim 1 wherein said polynucleotide encoding said negative selective marker is contained in a viral vector.

6. The method of Claim 5 wherein said viral vector is a retroviral vector.

7. The method of Claim 1 wherein said genetically engineered T-cells are administered in an amount of from about  $10^5$  cells/kg to about  $10^9$  cells/kg.

8. The method of Claim 3 wherein said interaction agent is ganciclovir.

9. The method of Claim 8 wherein said ganciclovir is administered in an amount of from about 2 mg/kg/day to about 10 mg/kg/day.

10. The method of Claim 9 wherein said ganciclovir is administered in an amount of about 10 mg/kg/day.

11. A method of treating a disease or disorder in a host wherein treatment of said disease or disorder in the host includes ablating the bone marrow of said host and administering to said host a T-cell depleted bone marrow transplant, said method comprising:

- (a) ablating the bone marrow of said host;
- (b) administering to said host a T-cell depleted bone marrow transplant;
- (c) administering to said host T-cells genetically engineered to include a polynucleotide encoding a negative selective marker; and
- (d) administering to said host, prior to the occurrence of graft-versus-host disease, an interaction agent, said interaction agent being administered in an amount effective to kill genetically engineered T-cells capable of providing a graft-versus-host effect in said host through interaction of said interaction agent with said negative selective marker.

12. The method of Claim 11 wherein said negative selective marker is selected from the group consisting of

thymidine kinases, xanthine-guanine phosphoribosyl transferase, and cytosine deaminase.

13. The method of Claim 12 wherein said negative selective marker is selected from the group consisting of Herpes Simplex Virus thymidine kinase, cytomegalovirus thymidine kinase, and varicella-zoster virus thymidine kinase.

14. The method of Claim 13 wherein said negative selective marker is Herpes Simplex Virus thymidine kinase.

15. The method of Claim 11 wherein said polynucleotide encoding said negative selective marker is contained in a viral vector.

16. The method of Claim 15 wherein said viral vector is a retroviral vector.

17. The method of Claim 11 wherein said genetically engineered T-cells are administered in an amount of from about  $10^5$  cells/kg to about  $10^9$  cells/kg.

18. The method of Claim 13 wherein said interaction agent is ganciclovir.

19. The method of Claim 18 wherein said ganciclovir is administered in an amount of from about 2 mg/kg/day to about 10 mg/kg/day.

20. The method of Claim 19 wherein said ganciclovir is administered in an amount of about 10 mg/kg/day.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/09040

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 48/00; C12N 15/09 15/85

US CL : 424/93.71; 435/172.3, 372.3

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.71; 435/172.3, 372.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN medline, embase, biosis, scisearch; APS

search terms: gene therapy, graft versus host disease, transplant?, lymphocyte?

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category <sup>a</sup>	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ORKIN, S.H. et al. Report and recommendations of the panel to assess the NIH investment in research on gene therapy. NIH Office of Public Information. 7 December 1995, pages 1-40, see entire document.	1-20
A	GALLOT, G. et al. Human HLA-specific T-cell clones with stable expression of a suicide gene: A possible tool to drive and control a graft-versus-host--graft-versus-leukemia reaction? Blood. 01 August 1996, Vol 88, No. 3, pages 1098-1103, see entire document.	1-20

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

<sup>a</sup>	Special categories of cited documents:	<sup>T</sup>	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principles or theory underlying the invention
<sup>*A*</sup>	document defining the general state of the art which is not considered to be of particular relevance	<sup>*X*</sup>	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
<sup>*E*</sup>	earlier document published on or after the international filing date	<sup>*Y*</sup>	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, each combination being obvious to a person skilled in the art
<sup>*L*</sup>	document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (to be specified)	<sup>*A*</sup>	document member of the same patent family
<sup>*O*</sup>	document referring to an oral disclosure, use, exhibition or other means		
<sup>*P*</sup>	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 24 JUNE 1997	Date of mailing of the international search report 20 AUG 1997
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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/09040

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BONINI, C. et al. Transfer of the HSV-TK gene into donor peripheral blood lymphocytes for in vivo immunomodulation of donor anti-tumor immunity after allo-BMT. Blood. 1994, abstract 427.	1-20
Y	SERVIDA, P. et al. Gene transfer into peripheral blood lymphocytes for in vivo immunomodulation of donor anti-tumor immunity in a patient affected by EBV-induced lymphoma. Blood. November 1995, abstract 843.	1-20